UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
CHEMICAL SAFETY
AND POLLUTION
PREVENTION

MEMORANDUM

DATE: June 30, 2011

SUBJECT: Efficacy Review for NP 4.5 (D&F) Detergent/Disinfectant;
EPA Reg. No. 1839-95;
DP Barcode: D387539

FROM: Lorilyn M. Montford
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APPLICANT: Stepan Company
22 West Frontage Road
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FORMULATION FROM LABEL:

Active Ingredient(s)	% by wt.
Alkyl (60% C_{14}, 30% C_{16}, 5% C_{12}, 5% C_{18}) dimethyl benzyl ammonium chlorides	2.25%
Alkyl (68% C_{12}, 32% C_{14}) dimethyl ethylbenzyl ammonium chlorides	2.25%
Inert Ingredients	95.50%
Total	100.00%

I BACKGROUND

The product, NP 4.5 (D&F) Detergent/Disinfectant (EPA Reg. No. 1839-95), is an EPA-approved disinfectant (bactericide, fungicide, virucide), mildewstat, and deodorizer for use on hard, non-porous surfaces in household, commercial, institutional, industrial, food processing, farm, animal care, and hospital or medical environments. The applicant requested to amend the registration of this product to add new claims for effectiveness as a disinfectant against *Klebsiella pneumonia* New Delhi Metallo-Beta Lactamase (NDM-1) Carbapenem Resistant and Canine parvovirus. The label states that the product is effective in the presence of 5% serum contamination. Studies were conducted at MICROBIOTEST, located at 105 Carpenter Drive in Sterling, VA 20164.

This data package contained a letter from the applicant's representative to EPA (dated February 28, 2011), EPA Form 8570-35 (Data Matrix), two studies (MRID 483996-01 and 483996-02), Statements of No Data Confidentiality Claims for both studies, and the proposed label.

Note: The laboratory report assigned MRID 483996-02 describes a study conducted for the product, Veterinarian Type Disinfectant (EPA Reg. No. 1839-100). The letter to EPA (dated February 28, 2011) states that the tested product, Veterinarian Type Disinfectant is a double-strength variant of the product, NP 4.5 (D&F) Detergent/Disinfectant, which is the subject of this efficacy report.

II USE DIRECTIONS

The product is designed to be used for disinfecting hard, non-porous surfaces, including: appliance exteriors, barber/salon instruments/tools, bathroom fixtures, bathtubs, bed frames, cabinets, cages, carts, chairs, coolers, counter tops, desks, door knobs, exercise equipment, exercise mats, floors, flower buckets, garbage cans and garbage pails, hospital beds, hospital equipment, inflatable plastic structures, kennels, mirrors, outdoor furniture, personal protective safety equipment, picnic tables, racks, shelves, shower curtains, shower stalls, sinks, tables, telephones, toilets, urinals, vanity tops, walls, whirlpools, and windows. The proposed label also indicated that the product may be used on hard, non-porous surfaces, including: glass, glazed ceramic, glazed porcelain, glazed tile, metal (e.g., chrome, stainless steel), plastic, sealed granite, sealed marble, and vinyl. Directions on the proposed label provided the following information regarding preparation and use of the product as a disinfectant: Add 2 ounces of the product per gallon of water (a 1:64 dilution) or 8 ounces of the product per gallon of water to inactivate Canine parvovirus (a 1:16 dilution). Apply the use solution with a mop, cloth, sponge, or sprayer. Wet all surfaces thoroughly. Allow surfaces to remain wet for 10 minutes. Remove excess liquid. For heavily soiled areas, a pre-cleaning step is required.

III AGENCY STANDARDS FOR PROPOSED CLAIMS

<u>Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments (Additional Bacteria)</u>

Effectiveness of disinfectants against specific bacteria other than those named in the AOAC Use-Dilution Method, AOAC Germicidal Spray Products as Disinfectants Method, AOAC Fungicidal Test, and AOAC Tuberculocidal Activity Method, must be determined by either the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products as Disinfectants Method.

Ten carriers must be tested against each specific microorganism with each of 2 product samples, representing 2 different product lots. To support products labeled as "disinfectants" for specific bacteria (other than those bacteria named in the above test methods), killing of the specific microorganism on all carriers is required.

Virucides

The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants) must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level.

Supplemental Claims

An antimicrobial agent identified as a "one-step" disinfectant or as effective in the presence of organic soil must be tested for efficacy with an appropriate organic soil load, such as 5 percent serum.

IV COMMENTS ON THE SUBMITTED EFFICACY STUDIES

1. MRID 483996-01 "AOAC Use Dilution Test Using *Klebsiella pneumoniae* New Delhi Metallo-Beta Lactamase (NDM-1) Carbapenem Resistant" for NP 4.5 (D&F) Detergent/Disinfectant, by Angela L. Hollingsworth. Study conducted at MICROBIOTEST. Study completion date – September 30, 2010. Amended final report date – February 18, 2011. Laboratory Project Identification Number 123-364.

This study was conducted against *Klebsiella pneumoniae* New Delhi Metallo-Beta Lactamase (NDM-1) Carbapenem Resistant (Clinical Isolate No. 10002; received from the Centers for Disease Control and Prevention (CDC), Atlanta, GA). Two lots (Lot Nos. 3533-96 and 3533-97) of the product, NP 4.5 (D&F) Detergent/Disinfectant, were tested using the AOAC Use-Dilution Method as described in the AOAC Official Methods of Analysis, 18th Edition, 2006. Use solutions were prepared by adding 2 parts of the product and 126 parts of sterile deionized water (a 1:64 dilution). A culture of the challenge microorganism was prepared. Non-heat inactivated horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed for 15 minutes in a 48-54 hour old suspension of test organism, at a ratio of 20 carriers per tube of 20 mL broth. The carriers were dried for 25 minutes at 37±2°C. Each carrier was placed in 10 mL of the use solution for 10 minutes at 20°C. The tubes containing the use solution were swirled after

addition of the carriers. Following exposure, individual carriers were transferred to Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were swirled gently after addition of the carriers (which differs from the AOAC methods specification of shaking the tubes thoroughly). All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, confirmation of the challenge microorganism, and antibiotic resistance.

Note: Antibiotic resistance of *Klebsiella pneumoniae* New Delhi Metallo-Beta Lactamase (NDM-1) Carbapenem Resistant was verified on a representative culture. An individual Mueller Hinton Agar was streaked with the prepared culture in a crosshatch pattern. After crosshatching, three equally spaced antibiotic disks were added to the plate. The plate was incubated and, following incubation, each zone of inhibition was measured and documented. The measured zones of inhibition (i.e., 0 mm for all disks) confirmed antibiotic resistance of *Klebsiella pneumoniae* New Delhi Metallo-Beta Lactamase (NDM-1) Carbapenem Resistant to penicillin, ceftazidime and gentamicin. See pages 9, 10, and 17 of the laboratory report.

2. MRID 483996-02 "Virucidal Efficacy Test Canine parvovirus" for Veterinary Type Disinfectant, by M. Khalid Ijaz. Study conducted at MICROBIOTEST (previously known as MICROBIOTEST, INC.). Study completion date – June 29, 2004. Laboratory Project Identification Number 123-170.

This study was conducted against Canine parvovirus type 2 (Strain CPV-2b/Eu; obtained from American BioResearch Laboratories), using A72 cells (obtained from American BioResearch Laboratories) as the host system. Two lots (Lot Nos. 2761-38 and 2761-40) of the product, Veterinary Type Disinfectant (EPA Reg. No. 1839-100; a 2x strength formulation of NP 4.5 (D&F) Detergent/Disinfectant), were tested according to a MICROBIOTEST protocol titled "Virucidal Efficacy Test Canine parvovirus," dated February 24, 2004 (copy provided). Use solutions were prepared by adding 4 parts of the product and 124 parts of sterile deionized water (a 1:32 dilution). The stock virus culture contained at least a 5% organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked bottoms of separate sterile glass Petri dishes. The virus films were dried for 30-60 minutes at room temperature. For each lot of product, separate dried virus films were exposed to 2.0 mL of the use solution for 10 minutes at 21°C. Following exposure, the plates were neutralized with 2.0 mL of fetal bovine serum. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl S-1000 columns, and diluted serially in Eagle's Minimum Essential Medium with 10% fetal bovine serum. A72 cells in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The inoculum was allowed to adsorb for 90-120 minutes at 37±2°C with 5±1% CO_2. Following adsorption, the cultures were incubated for 5-7 days at 37±2°C in 5±1% CO_2. Following incubation, the cultures were examined for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, cytotoxicity-related viral interference, and neutralizer effectiveness. The 50% fluorescent focus forming unit dose per mL ($FFFUD_{50}$/mL) was determined using the method of Reed and Muench.

V RESULTS

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested		Carrier Counts (CFU/ carrier)
		Lot No. 3533-96	Lot No. 3533-97	
483996-01	*Klebsiella pneumoniae* New Delhi Metallo-Beta Lactamase (NDM-1) Carbapenem Resistant	0/10	0/10	1.9×10^6

MRID Number	Organism	Results			Plate Recovery Control
			Lot No. 2761-38	Lot No. 2761-40	
483996-02	Canine parvovirus	10^{-2} to 10^{-3} dilutions	Cytotoxicity	Cytotoxicity	$\geq 10^{6.77}$ $FFFUD_{50}$/ mL
		10^{-4} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$FFFUD_{50}$ mL	$\leq 10^{3.50}$	$\leq 10^{3.50}$	
		Log reduction	≥ 3.27 $\log_{10}$	≥ 3.27 $\log_{10}$	

VI CONCLUSIONS

1. The submitted efficacy data (MRID 483996-01) support the use of a 1:64 dilution of the product, NP 4.5 (D&F) Detergent/Disinfectant, as a disinfectant with bactericidal activity against *Klebsiella pneumoniae* New Delhi Metallo-Beta Lactamase (NDM-1) Carbapenem Resistant on hard, non-porous surfaces in the presence of a 5% organic soil load for a 10-minute contact time. Complete killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralizer effectiveness testing showed positive growth of the microorganism. Viability controls were positive for growth. Sterility controls did not show growth.

2. The submitted efficacy data (MRID 483996-02) support the use of a 1:32 dilution of the product, Veterinarian Type Disinfectant (equivalent to a 1:16 dilution of the product, NP 4.5 (D&F) Detergent/Disinfectant), as a disinfectant with virucidal activity against Canine parvovirus on hard, non-porous surfaces in the presence of a 5% organic soil load for a 10-minute contact time. A recoverable virus titer of at least 10^4 was achieved. Cytotoxicity was observed in the 10^{-2} and 10^{-3} dilutions. Complete inactivation (no growth) was indicated in all higher dilutions tested. At least a 3-log reduction in titer was demonstrated beyond the cytotoxic level.

VII RECOMMENDATIONS

1. The proposed label claims that a 1:64 dilution of the product, NP 4.5 (D&F) Detergent/Disinfectant, is an effective disinfectant against *Klebsiella pneumoniae* New Delhi Metallo-Beta Lactamase (NDM-1) Carbapenem Resistant on hard, non-porous surfaces in the presence of 5% serum contamination for a 10-minute contact time. This claim is acceptable as it is supported by the submitted data.

2. The proposed label claims that a 1:16 dilution of the product, NP 4.5 (D&F) Detergent/Disinfectant, is an effective disinfectant against Canine parvovirus on hard, non-porous surfaces in the presence of 5% serum contamination for a 10-minute contact time. This claim is acceptable as it is supported by the submitted data.

3. The following revision to the proposed label is recommended:

- On page 13 of the proposed label, change "Feline Phinotracheitis" to read "Feline rhinotracheitis."